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The effect of genetic variation in chemokines and their receptors on HIV transmission and progression to AIDS

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Summary: The pivotal discovery that two chemokine receptors, CCR5 and CXCR4, serve along with the T-cell receptor-interacting CD4 molecule as the principal co-receptors for HIV-1 entry stimulated a search for common genetic polymorphism in their genes which might affect the course of AIDS. Four mutational variants, *CCR5-Δ32*, *CCR5-P1*, *CCR2-64I* and *SDF1-3'A* were discovered to play a regulatory role in HIV-1 infection, in the rate of progression to AIDS or both. Plausible physiological mechanisms to explain the population genetic association by these alleles have been advanced and are discussed critically here. Genetic ablation of AIDS progression by chemokine receptor and ligand gene variants has catalyzed development of novel therapies targeting the virus–co-receptor interaction. The functional and therapeutic implications of these AIDS restriction genes for disease progression and intervention are explored in this review.

Introduction

The scourge of AIDS has swept the world at a geometric rate of increase since its first discovery among homosexual men and recipients of HIV-contaminated blood products in the early 1980s (1–3). The virus has claimed some 14 million casualties, and today over 33 million people live with HIV infection (throughout this review we refer to HIV-1 as HIV), an agent for which there is no vaccine, and therapies that at best only delay AIDS (4). In 1996, hope appeared in two related areas. First, the introduction of highly active antiretroviral therapy (HAART) showed that combination inhibitors of the HIV protease and reverse transcriptase enzymes reduced viral replication to below detectable levels in the blood of most patients (5, 6). Unfortunately, HAART cannot eliminate HIV completely; quiescent virus reservoirs remain sequestered in protected compartments of infected patients for many years, only to rebound upon cessation of the powerful drug treatments (7). Furthermore, the AIDS-slowing drugs are unaffordable in countries of the less developed world, where they are most needed.

The second advance was the discovery that chemokine receptors, primarily CCR5 and CXCR4, serve as co-receptors,

along with the T-cell recognition molecule CD4, as entry portals for HIV infection (8–14). Over 90% of primary HIV infections involve what are termed M-tropic or R5-tropic strains, which readily infect CD4+ T lymphocytes, macrophages and monocytes *in vitro*. An initial docking step with CD4 triggers an HIV-envelope conformational change to enable gp120 to bind to CCR5 and initiate viral gp41-mediated virus–cell fusion (15, 16). The virus replicates efficiently in CD4+/CCR5+-bearing cell types: macrophages, monocytes, and T cells of lymph nodes, particularly in the intestine and colon (17–20), producing some billions of virions per day throughout the typical 10-plus years course of infection (21, 22). Most patients infected with subtype B HIV strains (the predominant strains in the US and Europe) experience a mutational transition in their HIV envelope gene which alters the cell tropism to permit CXCR4 utilization (X4- or T-cell-tropic preference) so that the mutated virus can now replicate in CXCR4-bearing cells, including immortalized T-cell lines *in vitro*. This increase in the prevalence of T-tropic HIV strains usually precedes an abrupt decline in CD4-bearing lymphocytes, the hallmark of AIDS onset (23, 24).

A number of excellent reviews describe the details of AIDS pathogenesis (25–27). Here, we shall concentrate on what genetic inferences can be derived from chemokine and receptor allelic polymorphisms, and we will illustrate outstanding questions that emanate from the human genetic approach to the AIDS pathogenic process. As was learned from genetic studies in mouse, *Drosophila* and other species, point mutations in structural and regulatory loci connected to disease phenotypes are interpreted in the context of empirical results to forge a clearer understanding of the interaction of HIV with the human organism. That virus–host interplay should be considered as a diverse ecosystem of differential tissue and development compartments which either allow the virus to replicate or which limit virus spread. Of particular note is that HIV incapacitates the very system evolved to destroy it, the cellular immune system, coordinated by the CD4+ T-helper cells in which the virus replicates.

A role for chemokines and their receptors in HIV cell entry was revealed by several studies that appeared in 1995 and 1996. Cocchi et al. (14) identified factors, secreted from CD8+ T cells, that blocked HIV-1 infection to be the CC chemokines, RANTES, macrophage inflammatory factor (MIP)-1α and MIP-1β, ligands of the soon to be identified chemokine receptor CCR5. Shortly thereafter, Feng et al. (8) demonstrated that a large human gene encoding an orphan chemokine receptor termed fusin (later renamed CXCR4) conferred HIV infectability on mouse cells already transfected with human CD4. These two seminal observations stimulated a flurry of confirmatory studies that established CCR5 and CXCR4 as the principal co-receptors, with CD4, for HIV infection through specific envelope binding, fusion and cell invasion (9–13, 28).

Common genetic mutations were rapidly discovered in coding and regulatory genes specifying the HIV co-receptors (CD4, CCR5, CXCR4), their ligands and other less efficient chemokine receptors for R5 and X4 HIV strains (29–40). These genetic polymorphisms were evaluated in AIDS epidemiologic cohorts (an AIDS cohort is a group of HIV-exposed or -infected patients followed clinically throughout the course of disease) for distortions in population genetic equilibria associated with clinical outcomes. Although multiple clinical parameters vary in AIDS epidemiological cohorts, our studies concentrated on five explicit clinical endpoints as potential sites of influence for genetic polymorphisms. Thus, we sought genetic associations that influence: 1) whether HIV-exposed individuals became infected or resisted infection; 2) the rate of progression to clinical AIDS after infection; 3) the actual AIDS-defining disease developed (e.g. Kaposi's sarcoma, *Pneumocystis carinii* pneumonia, neurological pathology, lymphoma, cytomegalovirus and other diseases); 4) the cellular and humoral immune response to HIV; and 5) the success or failure of HAART in reversing the course of AIDS.

The first AIDS restriction allele, *CCR5-Δ32*, was discovered in 1996 and shown to confer near absolute resistance to HIV infection among HIV-exposed individuals homozygous for the variant (29, 32–34). Additional variants in the upstream regulatory region of CCR5 (35–38), in the coding region of another chemokine receptor CCR2 (36,39), and in the 3' untranslated region of the transcript for stromal derived factor (SDF)-1 (40), the chemokine ligand for CXCR4, were also shown to regulate the rate of AIDS progression in cohort studies (*Table 1*). Affirmation of these genetic influences on HIV infection and AIDS outcomes improved our primitive understanding of the critical, almost collaborative, role that host cellular machinery exerts on the steady march to AIDS (25–28, 41). In this review, we concentrate on the implications that these mutational variant effects have for our understanding of how HIV destroys the immune system of infected individuals. We emphasize some significant uncertainties that remain to be resolved.

The *CCR5-Δ32* variant and AIDS

A common genetic variant in the coding region of the *CCR5* structural gene involves a 32 base pair deletion (*CCR5-Δ32*) that shifts the open reading frame to create a truncated protein. This

***Table 1*. Chemokine receptor and chemokine genes[a] that affect HIV-1 infection, AIDS progression and AIDS outcome**

Gene	Allele	Mode	Effect	Time	Citation
1) *CCR5*	*Δ32*	Recessive	Prevent infection	------	(29, 32–34)
CCR5	*Δ32*	Dominant	Prevent lymphoma	Late	(58)
CCR5	*Δ32*	Dominant	Delay AIDS	Overall	(29, 33, 34)
2) *CCR5P*	*P1*	Recessive	Accelerate AIDS	Early	(35–38)
3) *CCR2*	*64I*	Dominant	Delay AIDS	Overall	(39)
4) *SDF1*	*3'A*	Recessive	Delay AIDS	Late	(40)

[a]The chemokine ligands for CCR5 are RANTES, MIP1-α, and MIP1-β, variants of which have not been convincingly associated with AIDS (but see (130)). SDF-1 is the single known natural ligand for CXCR4.

protein fails to reach the cell surface in individuals homozygous for the variant (32, 34). CCR5-+/Δ32 heterozygotes have reduced levels of quantifiable CCR5 receptors on their cell surface, notably rather greater than the expected 50% reduction due to the gene dosage effect (42–44). The mean reduction to 20–30% of wild-type levels in *CCR5*-+/Δ32 heterozygotes is perhaps because nascent CCR5-Δ32 polypeptides dimerize with their wild-type CCR5 counterparts in the endoplasmic reticulum, retarding the transport of CCR5 to the cell surface (43).

Genetic association analysis of over 10,000 individuals at risk for HIV infection has shown that *CCR5*-Δ32/Δ32 homozygotes completely resist infection by primary R5-tropic HIV strains (41), although there are a few reports of homozygotes who have become infected with the later stage X4 strains, likely because the virus has surpassed the requirement for CCR5 by utilizing CXCR4 instead (45–49). It may be important that rare homozygotes who harbor X4 strains have a lower HIV viral load (i.e. concentration in blood) than do *CCR5*+/Δ32 or *CCR5*+/+ individuals infected with R5 or R5X4 viruses (48). If affirmed, this differential may reflect limits on the ability of CXCR4+ cells to replicate HIV maximally *in vivo*. It could explain, at least partially, why R5 viruses are so favored in early infection, estimated at 90–95% of primary infections (50–54). A limited capacity of CXCR4+CCR5– cells to replicate HIV may also explain the shift from X4 to R5 predominance observed in two *CCR5*-+/+ individuals soon after primary infection, since an excess of virus production from CCR5+CD4+-activated lymphocytes would select *in vivo* for a preponderance of R5 HIV variants, although other explanations are also possible (55).

X4 viruses have been reported to be more cytopathic, at least *in vitro*, than their R5 counterparts, leading to speculation that X4 viruses are also more virulent or cytopathic to CD4+ T cells *in vivo* (26). Such observations could help explain why a rapid collapse of CD4+ T-cell populations occurs after the X4 viruses become predominant, although differentials in the efficiencies of X4 and R5 viral replication or their effect on CD4 T-cell production may also have an influence. Complicating any interpretation is the demonstration that R5 simian immunodeficiency virus (SIV) strains are certainly pathogenic and lethal without the X4 transition (as R5 HIV strains can be in people); SIV strains can actually increase their virulence over the course of infection without any switch in co-receptor usage (56). A clear rationale for the R5–X4 transition remains elusive, although the remarkable transition in tropism in a significant subset of individuals is firmly established (23–26).

Heterozygotes (*CCR5*-+/Δ32) are readily infected with HIV in patient populations. However, once infected they show a 2–3 year delay in the time it takes to develop AIDS-defining pathology (29, 33, 57). They also display a reduction in viral load, which lends support to the simple explanation that the fewer available CCR5 portals on cells of *CCR5*-+/Δ32 heterozygotes retards HIV replication and the virus-mediated destruction of the CD4+, CCR5+ T-cell lymphocyte population. These epidemiologic genetic findings are rather important because they affirm the notion that CCR5-mediated cell entry can be rate limiting, even in patients with a single copy of the wild-type gene. That natural genetic mutations can slow or delay AIDS in patient populations raises the prospect of therapeutic intervention targeting the virus co-receptor interaction (see below).

The development of specific AIDS-defining diseases also seems to be regulated by CCR5 mutations. In a large case control study, *CCR5*-+/Δ32 heterozygotes infected with HIV were only half as likely to develop non-Hodgkin's B-cell lymphoma as were *CCR5*-+/+ individuals (58). B cells express CCR5 on their surface and are stimulated by RANTES, suggesting that HIV and B cells may interact directly in lymphoma incidence among AIDS patients. A reduction in the number of B-cell CCR5 receptors may decrease B-cell responses to mitogenic

stimulus by CCR5 ligands. However, there are many B-cell dysfunctions in the hyperstimulated immune systems of HIV-infected people, with hypergammaglobulinemia being common. B-cell activation is rapidly reversed by HAART, suggesting a relationship between HIV replication and immune activation (59).

A puzzling aspect of the *CCR5* genetic restriction involves the population distribution of the variant and its inferred natural history. The *CCR5*-Δ32 allele is common among European Caucasians (allele frequency 5–15%), but virtually absent among native African and East Asian ethnic groups (*Table* 2) (41, 60–64). Population genetic assessment of the length of linkage disequilibrium segments around the *CCR5* locus on chromosome 3 in European Caucasian populations indicates that the *CCR5*-Δ32 mutation occurred just once on a particular haplotype flanked by specific adjacent polymorphic short tandem repeat (STR) locus alleles (*Fig. 1*) and that it arose recently, probably less than 4,000–6,000 years ago (60, 61). Further, the most recent frequency increase of the *CCR5*-Δ32 haplotype region occurred in human populations around 700 years ago (60). Since human populations have been rather large (10,000–100,000) for over 50,000 years, these results all indicate that the frequency of the *CCR5*-Δ32 allele, a human "knockout" variant, rose rapidly in Caucasian European populations in a very short time. Such a rapid hike in allele frequency can only be explained by a strong, continuous, selective pressure which reproductively favored the carriers of *CCR5*-Δ32.

The cause of the strong selective pressure is not obvious, but the coalescent dating results (~700 years ago) raise the possibility that the Black Death (bubonic plague) of 14th Century Europe is an epidemic candidate, particularly since plague ravaged Caucasian populations each generation for six centuries prior to the Black Death. Similarities in target cells and tissues (macrophages, lymphocytes) of AIDS and the plague agent *Yersinia pestis* raise the question of whether *CCR5* plays an adverse role in the survival of people infected with the plague bacillus. A functional connection of *CCR5* and *Yersinia* has not been demonstrated to date, but neither have negative results excluded it. One report has linked *CCR5* murine gene knockouts with the immune system's efficiency in clearing an intracellular mycoplasma, *Cryptococcus neoformans*, from neural tissue (65). Another showed that rabbit myxoma (pox) virus effectively utilizes CCR5 for entering cells, raising the prospect that historic epidemics of smallpox could be candidates for selective favoring of *CCR5*-Δ32 (66). Finally, there are also recent findings that associate *CCR5*-Δ32 with hypertension (67).

Screens of the *CCR5* gene for mutations other than Δ32 also imply that the receptor has been the object of natural selective pressure, probably by infectious disease in the past. Twenty-two distinct alleles of *CCR5*-coding genes have been described to date (68–71). Eighteen of these (82%) are non-synonymous (amino acid altering from the common wild-type allele) (*Fig. 2*), a rather high incidence compared to other known coding gene polymorphisms (72). This high level of codon-altering variants is a signal of selective pressure to retain amino acid diversity, as has been attributed, for example, to alleles at the mammalian major histocompatibility complex (MHC) (72). The parallels with MHC in polymorphism pattern, combined with the historic inference discussed above, make a provocative case that the *CCR5* allele products have been co-opted frequently by other infectious diseases that would confer selective advantage to the carriers of mutational variants (60, 63, 66, 69, 70).

***Table 2.* Allele frequencies of AIDS restriction genes in major human ethnic groups**

Gene	Allele	European Caucasians	Africans	East Asians
CCR5	+	0.86–0.96	1.0	1.0
	Δ32	0.044–0.14	0.0	0.0
CCR2	+	0.90	0.77	0.75
	64I	0.10	0.23	0.25
SDF1	+	0.79	0.98	0.74
	3'A	0.21	0.02	0.26
CCR5P	*P1*	0.56		0.44
	P2	0.09		0.23
	P3	0.14		0.15
	P4	0.35		

CCR2-64I

CCR2 is one of over a dozen identified chemokine co-receptors that can also serve, albeit weakly, as HIV co-receptors, and it is CCR5's closest genomic relative based on chromosomal proximity (*Fig. 1*) and DNA sequence homology (13, 28, 41, 74). A common variant, *CCR2-64I*, which substitutes an isoleucine for a valine in the first transmembrane domain of CCR2, causes a delay in the onset of AIDS for homozygotes and heterozygotes, although it has no effect on HIV transmission (39). The epidemiological effect on AIDS progression was surprising given the innocuous change (val–ile) in a position buried in one of the seven transmembrane segments of this receptor. Yet, several independent cohort studies have affirmed the AIDS-delaying effects of *CCR2-64I* (36, 38, 75–77). Our initial suspicion that the mutation was tracking *CCR5*-Δ32 by linkage disequilibrium (*CCR5* and *CCR2* are 14 kb apart, see *Fig. 1*) was refuted since

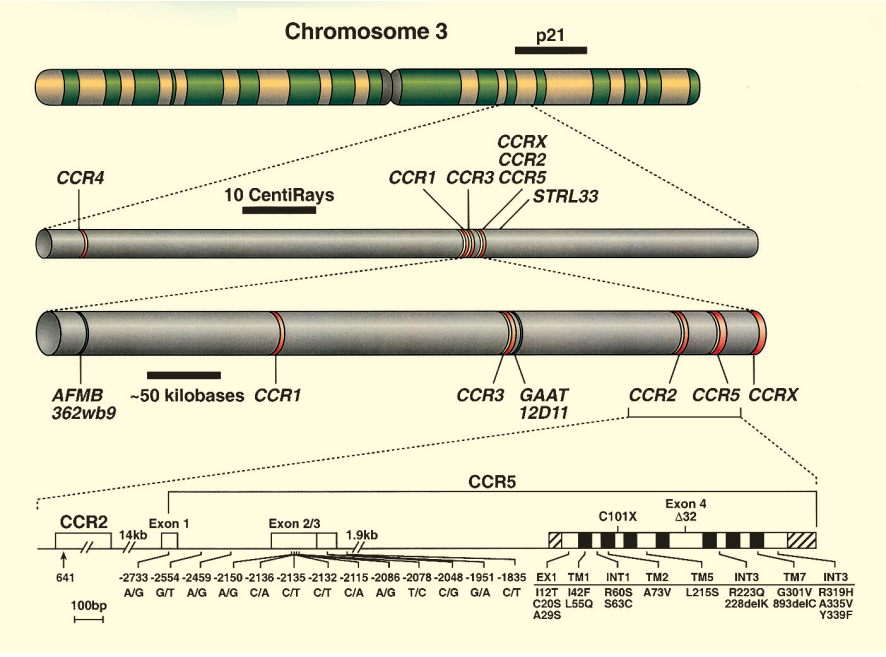

Fig. 1. Genetic map of chemokine receptor gene cluster on human chromosome 3p21. CCR2-64I, CCR5-Δ32 and single nucleotide variants of the CCR5 promoter region are indicated by their nucleotide position (29, 60, 68).

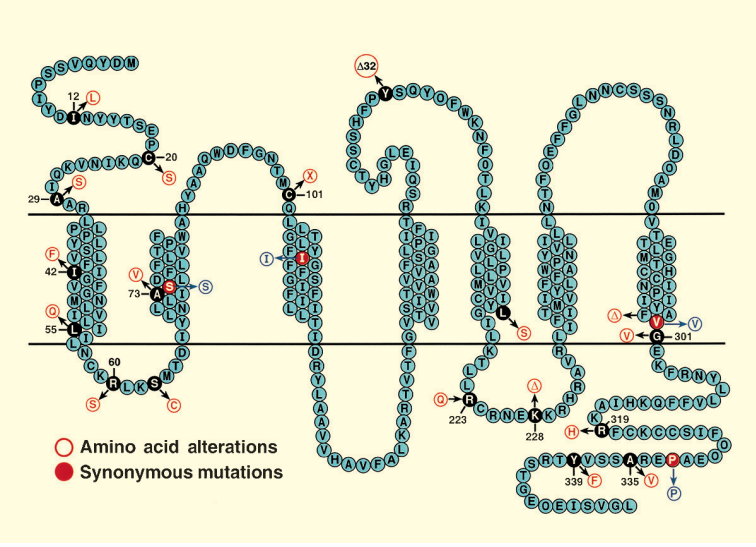

Fig. 2. Diagram of a CCR molecule spanning a cell membrane with single nucleotide polymorphisms and amino acid alterations indicated (67–70).

CCR2-64I-bearing chromosomes invariably carried CCR5+ alleles while CCR5-Δ32 chromosomes were always CCR2+ (39). Thus, CCR5-Δ32 and CCR2-64I had independent and potent additive effects on delaying AIDS. Linkage disequilibrium with upstream regulatory alleles of the primary receptor CCR5 was also excluded as the explanation; both CCR5-Δ32 and CCR2-64I are invariably carried on a chromosome with a CCR5 promoter haplotype (CCR5P1, see below) which was associated with accelerated, not delayed, AIDS onset (35). Functional assays showed little difference in monocyte chemoattractant protein-1 ligand signaling or in the quantity of CCR2 expressed on peripheral blood mononuclear cells (PBMCs) of different CCR2 genotypes, but they did show indirect evidence for "cross talk" or heterologous desensitization between CCR2 signaling and the quantity of CXCR4 and CCR5 expressed (78, 79). A provocative but as yet unconfirmed report indicated that the CCR2-64I protein product can preferentially dimerize with the CXCR4 polypeptide, sequestering it in the endoplasmic reticulum, while the CCR2+ peptides do not (80). Such differential intracellular kinetics between CCR2 allele products and primary HIV co-receptors *in vivo* might reduce the rate of disease progression by limiting the number of available CCR5 and/or CXCR4 co-receptors and hence, indirectly, the rate of viral replication.

The distribution of CCR2-64I among different human ethnicities differs from CCR5 in that all major ethnic groups have appreciable frequencies of CCR2-64I (*Table* 2). Among a native

African cohort from Nairobi, the frequency of the *CCR2-64I* (f=0.23) allele was twice as high as in American Caucasians (f=0.10), and the delay in AIDS progression was twice as great (relative risk (RR)=4.17) as in similarly proportioned disease categories of Caucasian cohorts (RR=2.33) (63). The dramatic increases in both protective allele frequency and strength of genetic protection in the Nairobi cohort illustrate the striking influence of chemokine receptor-utilizing infectious agents on variant allele distribution. The apparent increase in *CCR2-64I* influence on AIDS in native Africans may be related in part to the absence of *CCR5-Δ32* from that population.

A fascinating evolutionary sidebar to these notions has emerged from a study of red capped mangabeys (81), a free-ranging subspecies of African mangabey. SIV is endemic among several small African monkey species, including mangabeys, and is thought to represent the origin of HIV-2, a phylogenetically divergent and less virulent strain of the AIDS virus which is restricted to west Africa (82). Genotypic sampling of the red capped mangabey revealed the presence of a CCR5 receptor-inactivating Δ24 deletion mutation with a frequency of 87%, meaning that 98% of individuals carried at least one copy of *CCR5-Δ24*. SIV isolates from other monkey species all use CCR5, but the red capped mangabey SIV isolate does not; its primary entry receptor is CCR2. It seems that the high frequency of genetic resistance (*CCR5-Δ24*) in this subspecies evolved as a selected protection against an historic pathogenic version of SIV. That host adaptation was subsequently answered by a directed viral tropism shift to utilize and prefer a new, heretofore minor, co-receptor, CCR2. Such observations reinforce our suspicion of the ongoing Darwinian natural struggle for survival between the genomes of pathogenic agents and their hosts (82, 83)

CCR5 promoter alleles

Although the *CCR5-Δ32* and *CCR2-64I* effects were verifiable and at least partially interpretable in a functional context, they are present in only a small fraction of HIV-exposed uninfected individuals (<20%), and they account for only a modest proportion of the variation in the AIDS outcome of infected patients (*Fig.* 3). We, and others, were curious as to whether regulatory mutations in the *CCR5* promoter region could be found which might affect HIV disease. To date there are 13 distinct single nucleotide polymorphisms (SNPs) within the 1,000 bp region upstream of *CCR5*-coding exons that exhibits promoter and regulatory activity (*Fig.* 1) (35–38, 68, 69, 84). These 13 SNPs can be theoretically assorted in 2^{13}=8,192 possible haplotype combinations. Thirteen haplotypes have actually been observed, four of which (designated *CCR5P1-P4*) are relatively common among Caucasian and African American populations (*Table* 3). Through complete linkage disequilibrium, *CCR5-Δ32* is invariably associated with *CCR5P1*, as is *CCR2-64I*. In addition, there was an appreciable frequency of *CCR5P1* alleles linked to the wild-type allele for both *CCR5* and *CCR2*. Thus, for genetic/epidemiologic purposes, there were six haplotypes to consider (*Table* 3) (these can be considered as "alleles" of the [*CCR2*, *CCR5P*, *CCR5*] superlocus). When Martin et al. (35) tested each haplotype allele (using dominant or recessive models for various infection and AIDS progression endpoints), they observed the expected *CCR5-Δ32* and *CCR2-64I*-mediated delay of AIDS, but also a more rapid descent to AIDS among HIV-infected individuals who were homozygous for *CCR6P1/P1* and wild type for *CCR5* and *CCR2* (*Fig.* 3). There was no apparent effect of different promoter haplotypes on the rate of HIV transmission (35, 36).

Table* 3. Haplotype allele frequencies including *CCR2-+, 64I; CCR5-P1-4; CCR5-+, Δ32

[*CCR2.CCR5P.CCR5*]	European Caucasian	African American
[+.P1.+]	0.36	0.26
[64I.P1.+]	0.10	0.16
[+.P1.Δ32]	0.10	0.02
[+.P2.+]	0.09	0.23
[+.P3.+]	0.001	0.19
[+.P4.+]	0.35	0.15

A plausible hypothesis for the *CCR5P1/P1* acceleration of AIDS progression would involve upregulation of *CCR5* gene transcription increasing available cell surface HIV co-receptors. However, quantitative analyses of: a) *CCR5* promoter-driven transcription of a luciferase gene-expressing construct; b) HIV infectivity with R5 or R5x4 dual-tropic HIV strains; or c) mean concentrations of cell surface CCR5 on PBMCs showed no difference among different promoter genotypes (35). These negative results should be interpreted cautiously, however, since the experiments would reveal only constitutive promoter differences and not cell-specific responses to transcription factors. In fact, oligonucleotides specific for alternative forms of one SNP site (−2,554), which defines the *CCR5P1* haplotype allele, show allele-specific recognition of nuclear transcription factors belonging to the cREL family (85). This implies that transcription factors in certain cell types might differentially bind variant promoter alleles, and so regulate *CCR5* transcription. However, differential transcription factor binding to this site (−2,554) cannot provide a full explanation for the association of *CCR5-P1/P1* with rapid progression to AIDS, because the

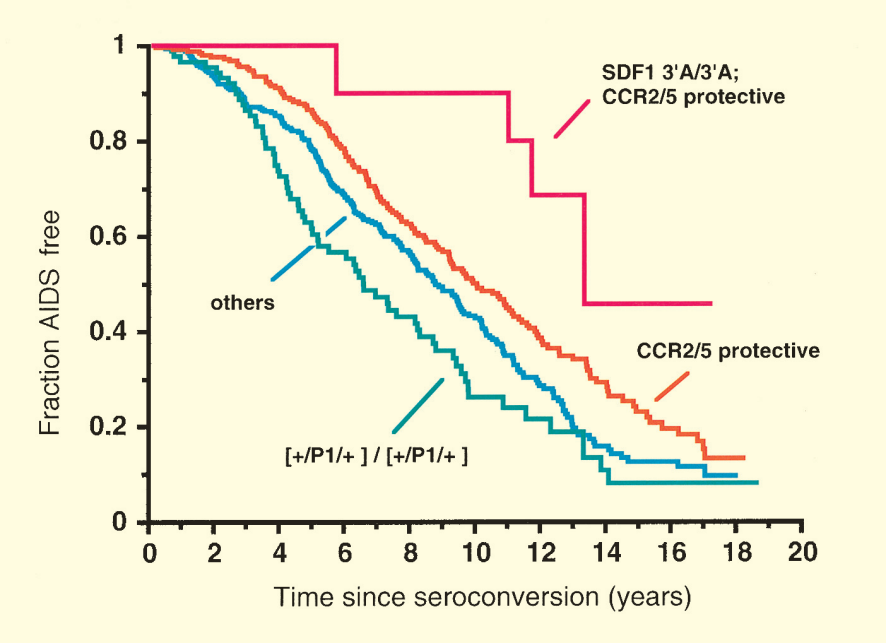

Fig. 3. Kaplan Meyer survival curves show differences in the rate of progression to AIDS (1993 CDC definition) over a 20-year interval among Caucasian seroconverters (patients with a known HIV infection date).
[Data from (29, 35, 39, 40)]

CCR5P1 SNP (–2,554) "G" is also found in *CCR5P2* (but not in *CCR5P3* and *CCR5P4*), an allele that does not accelerate AIDS (35, 85).

The *CCR5P1* promoter allele association was the first genetic variant to be associated with rapid progression, although other AIDS-accelerating variants of other genes have been observed more recently (86–89). The hypothesis that the genetic effect is mediated by an increase in available CCR5 portals is supported by the epidemiologic pattern. The strongest acceleration mediated by *CCR5P1/P1* occurs in the first 5 years of infection, a period when R5 virus predominates in 90–95% of patients (50-54). Finally, irrespective of *CCR5-Δ32* and *CCR2-64I*, it seems that between 10 and 17% of the rapid progressors who succumb to AIDS in less than 3.5 years after infection do so because they are homozygous for the *CCR5P1/P1* promoter allele (35).

SDF1-3'A

If variants of the CCR5 and CCR2 genes that encode R5-HIV strain receptors could limit AIDS, perhaps variants in the late stage X4-HIV receptor, CXCR4 or its ligand might as well. Polymorphism discovery screens of the *CXCR4* gene have to date yielded only two nucleotide variants which have little epidemiologic consequence (30, 31). When the coding region of the only known CXCR4 ligand, SDF, was interrogated, a common SNP variant at position 801 (counting from the AUG codon) in the 3' untranslated region (3'UTR) of a splicing variant transcript for SDF-1β was discovered (Fig. 4) (40). The variant is 37 base pairs from two DNA segments that are respectively 88% and 92% conserved in sequence between human and mouse *SDF-1* homologs (Fig. 4). This level of sequence conservation within a 3'UTR signals selective constraints on mutational divergence for the segment, such as a recognition sequence for RNA- or DNA-binding regulatory factors. HIV-infected individuals homozygous for the *SDF1-3'A/3'A* variant show a remarkable level of protection against AIDS in pooled or separated cohorts. Among individuals with both *SDF1-3'A/3'A* and *CCR5* (or *CCR2*) heterozygous protection, the protective effect is quite strong – several-fold higher than that conferred by *CCR5* or *CCR2* heterozygosity alone (Fig. 4). Indeed, no double protected individual in the Winkler et al. study progressed to AIDS until at least 10 years after infection (40), a remarkable statistic considering that roughly half of the genetically unprotected individuals succumbed to AIDS in fewer than 10 years post-infection.

The epidemiologic interaction of *CCR5/2* and *SDF1-3'A* (a genetic phenomenon termed epistasis) suggests that a functional interaction might explain the enhanced protection. One hypothesis is that *CCR2* and *CCR5* variants slow AIDS by limiting the number of CCR5 co-receptors that mediate the replication and spread of primary, early stage R5 HIV, while the *SDF1-3'A* variant restricts the emergence of X4 tropic HIV strains and the ensuing AIDS-accelerating process. A possible mechanism would be overproduction of SDF-1 in local compartments, which binds to and blocks the CXCR4 receptors required for X4 viruses to emerge and predominate. Direct evidence for an effect of *SDF1-3'A* on the synthesis, quantity or half life of the

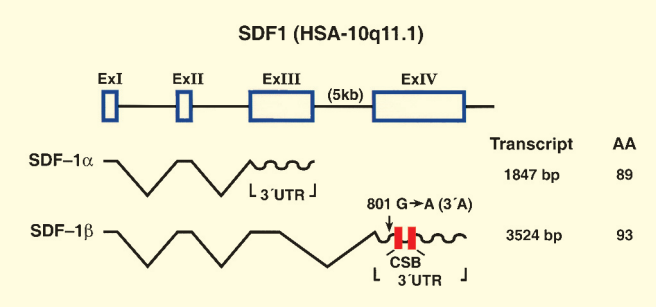

Fig. 4. Map of the SDF1 locus on human chromosome 10q11.1 with exons indicated, plus two alternative spliced transcripts α and β and position of SDF1-3'A variant in the 3'UTR of SDF1β transcript (132). Conserved sequence blocks (CSB) 37 bp downstream from *SDF1-3'A* are highly conserved in sequence (88% and 90%) between human and mouse 3'UTR regions. *SDF1-3'A* is located at position 801 counting from the AUG (40). CSB1 is position 918–953 and CSB2 is position 1037–1068.

ligand has not been obtained *in vitro* (90). Because SDF expression is limited to stromal cells and other tissues that are not easy to quantify (i.e. SDF is not expressed in PBMC or B-cell lines), this notion is difficult to test *in vivo* (91–93).

It should be mentioned that the *SDF1-3'A*-mediated protection, although replicated in separate cohorts from the NCI study (40), has been equivocal in other studies (38, 94–97). The explanation is uncertain, although the mixing of ethnic groups, the inclusion of seroprevalent patients, or the frailty (survival) bias of cohorts initiated some years after HIV infection might mask some epidemiologic signals (98–100). Also, the *SDF1-3'A/3'A* effect is recessive, so homozygotes are rare (~6%), a situation that demands large numbers of study participants to gain the required statistical power. We cannot exclude the possibility that the *SDF1-3'A* association with disease progression is not generalizable, although the observed strength of epidemiology protection observed in the NCI study is provocative and should not be overlooked (40).

Clinical consequences of AIDS restriction gene variants

The *CCR5-Δ32* variant abrogates *CCR5* gene function totally in homozygous individuals, who show few clinical symptoms of this loss of chemokine receptor function save for a suggested increased risk for hypertension (67). Homozygous individuals live healthy lives with little genetic cost (29, 33, 34, 67). Mice in which the *CCR5* gene has been knocked out are also born relatively healthy (101), perhaps because the CCR5 signaling function and ligand recognition is genomically redundant, i.e. other chemokine receptors back up the chemokine recognition and lymphocyte trafficking roles of CCR5 (74). Nevertheless, CCR5 does not seem to play and role in the reactive functioning of the murine immune system, as demonstrated by studies in CCR5 knockout mice. These animals have a greatly reduced survival after experimental infection of the brain with *Cryptococcus neoformans* (65); partial defects in the clearance of *Listeria donovani* (102); and an increased susceptibility to *Toxoplasma gondii*, due to decreased production of interleukin-12 and interferon (103). Only innocuous changes have been discovered in *CXCR4* or in *SDF1*-coding genes (30, 31, 40), while mouse knockouts for both CXCR4 and SDF1 are embryonic lethals (104–106). CXCR4-SDF1 signaling is physiologically and genomically unique and apparently indispensable, explaining the sensitivity of the genes to mutational tinkering. The *CCR2-64I* and *CCR5P1* variants do seem to alter functional interaction kinetics sufficiently to be detectable in our sensitive AIDS progression screens, but probably not enough to diminish immune functioning appreciably. If one wished to discover a gene defect that would point to therapeutic intervention for AIDS, the ideal would be a cellular host interaction that was required for AIDS progression, but completely dispensable for the individual. This is a precise description of CCR5 but it does not apply exactly to CXCR4 or SDF1.

Implications for AIDS therapy

The development of protease and reverse transcriptase inhibitors of HIV replication has had a major impact on the course of the AIDS epidemic in the developed world. It is now clear, however, that these drugs cannot eradicate HIV from infected individuals (7). Concerns about the long term side effects of protease inhibitors on some individuals (107) and the increasing transmission of drug-resistant variants (108) are other factors that emphasize the need to identify new classes of anti-HIV drugs able to suppress HIV replication efficiently.

The convincing connection of CCR5 and CXCR4 receptors to HIV infection and AIDS onset raised hopes for their exploitation in therapies that disrupted the earliest but continuing process of HIV cell infection and spread. Intervening in HIV–co-receptor interactions not only offers a novel cellular avenue for drug development, but also targets at least one critical function (CCR5) for AIDS progression which is dispensable for human health. Furthermore, seven transmembrane G protein receptors are familiar targets for pharmaceutical investigation, as they have been targeted extensively for treatment of ulcers, asthma, arthritis and psoriasis (109–111).

Several approaches to blocking HIV infection *in vitro* are promising and a few have entered clinical trials (*Table* 4). Detailed recent reviews describe promising strategies of drug development and nascent clinical trials (55, 109–113). The

Table 4. **Potential strategies for AIDS therapy which target HIV–co-receptor interaction**

	Reference
CCR5 and CXCR4 ligand derivative or small molecule antagonists that block HIV infection/replication but do not signal	117–121
Gene therapy – antisense, ribozymes	
"Intrakine" retention of CCR5 or CXCR4 on endoplasmic reticulum using endoplasmic reticulum-binding ligand attachment	125, 126
Immunological interference: monoclonal antibodies	122
Modulation of CCR5-bearing cells by antibody to CD3 and CD8	123, 124
Bone marrow transplants using *CCR5-Δ32/Δ32* donors	109, 111
Molecular antagonist of HIV-gp41 using D-peptides	114–116

See text and (55, 109–113) for discussion and further citations.

CCR5-Δ32-mediated genetic ablation of HIV infection has also stimulated the search for inhibitors that target the HIV envelope glycoproteins gp120 and gp41 (114, 115). Sites are known for both of these proteins at which peptides, natural compounds or small molecules can inhibit virus–cell attachment or the subsequent fusion process. One gp41-targeted fusion inhibitor, the T-20 peptide, has an antiviral effect in Phase I trials (116). Other targets for entry inhibitors include the co-receptors, of which CCR5 and CXCR4 are the most important (117). Small molecule and peptide-based compounds are known which antagonize HIV-1 entry via either CCR5 or CXCR4 (117–120). Some of these inhibitors will enter clinical trials this year. CCR5 expression levels, and hence the CCR5 genotype, are likely to influence the efficiency with which such antiviral therapies affect HIV replication. This is easy to imagine for specific therapies directed at blocking HIV entry via CCR5; the higher the level of expressed receptor, the more inhibitor should be needed to suppress its function as an HIV co-receptor. Given the range of CCR5 expression found in humans (it can vary by 20-fold among CCR5-+/+ individuals) (42–44), this could be a significant influence on the amount of the CCR5 inhibitor that must be given, especially if the inhibitor has a low affinity for CCR5.

The chemokine ligands of co-receptors are under consideration as antivirals (55, 109). However, these are agonists that affect the target cells for HIV-1 replication, which is an undesirable property for an antiviral. *In vitro*, both CC- and CXC-chemokines can significantly enhance HIV-1 replication under some conditions, and modified RANTES derivatives have been reported to promote the evolution of X4 isolates in a murine model system (121). This would not be beneficial if it occurred in infected humans. In addition, chemokines are not orally bioavailable and they have a very short half life *in vivo*. The practical obstacles to the clinical development of these compounds as antivirals are very real.

HIV co-receptor availability has also been diminished by using murine monoclonal antibodies against the receptors (122) or by downregulating CCR5 production and R5 HIV infection with monoclonals to CD3 and CD28 (123, 124). "Intrakine" co-receptor antagonists which attach chemokines to the endoplasmic reticulum and retard transport of their receptors to the cell surface have also been developed (125, 126). Gene therapy approaches using antisense or ribozyme constructs to limit CCR5 or CXCR4 expression are also effective *in vitro* (107). There is even some consideration of utilizing HIV-resistant CCR5-Δ32/Δ32 individuals as donors for bone marrow stem cell transplants to AIDS-lymphoma patients (109–111).

In addition to the deep insight into the process of AIDS pathogenesis that emanated from the co-receptor HIV studies plus the promise of novel therapies under development, there is another application of the genetic variants that deserves emphasis. As there is yet no effective vaccine or real cure for AIDS, the research community is seeking new drugs and potential vaccines suitable for human trials. The genetically heterogeneous nature of study populations needs to be considered when evaluating test agents in clinical trials. A portion of that heterogeneity will derive from the genotype (for AIDS resistance loci) of the study participants. Thus, genotypic assessment of volunteers in clinical trials might be invaluable in the interpretation of the trial outcome.

For example, in one recent study, primary HIV-1 isolates from patients who responded poorly to HAART were characterized (127). The isolates from HAART recipients who had only minor clinical symptoms despite being viremic used predominantly CXCR4 to enter primary cells (127). But how does the response to HAART vary with the CCR5 genotype? This is largely unknown, although there has been an oral report that CCR5-Δ32 heterozygotes responded to HAART (protease and reverse transcriptase inhibitors) better than CCR5 wild-type

individuals (128). Perhaps at the very low levels of HIV-1 replication found in individuals receiving HAART, the level of CCR5 expression on the target cells becomes more limiting for infection than it would when viremia levels are higher. Given the now widely accepted importance of T-cell production rates in determining the course of HIV infection (129), it may also be that the identity and quantity of HIV co-receptors expressed in sites of T-cell production could have a major influence on disease outcome. These sites can have thymic (130) or poorly understood extrathymic locations, perhaps including the gut (17–20). Any infection of T-cell precursors by HIV-1 could have a dramatic effect on CD4+ T-cell production over a prolonged period (129).

Conclusions

Research into the process and kinetics of HIV–co-receptor interaction and dependency has become a very active area of AIDS research. The attraction involves understanding a physiological process that limits the kinetics of HIV infection and AIDS pathogenesis. Genetic polymorphisms (*Table 1*) that limit these processes in the HIV-exposed populations validate the critical rate limiting steps *in situ* and point to new targets for therapeutic intervention. Besides providing insight into the pathogenic process and stimulating new therapeutic opportunities (*Table 4*), the genes can also assist in interpreting clinical trials of other agents such as antivirals or HIV vaccines by implicating host genetic influence on trial outcomes. Finally, the genes are also likely to be relevant to pharmacogenetics, the design of genotype-specific therapy for infected patients.

The advances achieved have unfulfilled potential for both therapy and prevention in what is now listed among the greatest infectious disease scourges in human history. Numerous uncertainties remain, but the combined tools of virology, cell biology and genetic epidemiology are revealing the secrets of this devastating human catastrophe.

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